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FACS ASSAY

Used by the laboratory of William C. Gause, Ph.D.

1. PREPARE SINGLE CELL SUSPENSION FROM MESENTERIC LYMPH NODES (MLN):

- Remove MLN from each mouse.
- Place on a cell strainer in culture dish with 5 mL cold RPMI + 2 % FCS, on ice (each mouse's MLN in separate dish).
- Keep tissue cold throughout this procedure.
- Gently grind each with a plunger against cell strainer (Becton Dickinson), submerged in cold media.
- Spin down at 1200 rpm for 7 min.
- Remove supernatants, wash the cells with RPMI + 2% FCS 2 times.
- Remove supernatant and resuspend the cells in 10 mL RPMI + 2% FCS.
- Filter through 100 μm sterile filter membrane (Labcor Products, Inc.) into orange-capped 15 ml tube.
- Count cell number and spin at 1200 rpm for 7 minutes.
- Pour supernatant down sink quickly.
- "Rack" to resuspend.
- Adjust cell concentration to 1 x 10^7/mL in cold RPMI + 10 % FCS.

2. DETERMINE NUMBER OF CELLS PER MILLILITER:

A. Coulter Counter Method:

- Turn on the power to the Coulter Counting Machine.
- Press “Functions” and put blank in.
- Press “Start” twice.
- Press “Setup.”
- Put cell size between 3.8 and 7.8 μm.
- Place sample in chamber.
- Press “Start” to count.

B. Trypan Blue Method:

- Use trypan blue diluted 1:10
- Dilute cells in trypan blue as appropriate, usually between 1:10 and 1:50
- Count the number of cells in five large boxes in hemocytometer (#)---------->>>(#) (2) (dilution factor)(1000)=number of cells per ml.

3. STAIN CELLS FOR FACS:

- Each stain requires 1 million cells per 100 μl (10 million cells per ml) in Sarstedt 55.526 5 ml tubes
- Pool cells from each group if necessary, preferably using equal numbers of cells from each mouse
- Wash with DPBS + 0.1 % NaN_3 + 2% FCS
- Remove supernatants and suspend cells in 100μL FACS buffer (DPBS + 0.1% NaN_3 + 2% FBS)
- Add 1 μg blocking antibody 24G2 to each tube
- As appropriate, add the first antibodies, for example: (Titrations are necessary to determine the appropriate quantity to add)
  - IL-2 biotin
  - GK1.5 CD4-FITC
  - B220-cychrome
  - MHCII-biotin
- Incubate 0.5 hours on ice
- Add 1 ml DPBS + 0.1 % NaN_3 + 2% FBS, spin, dump, rack
- For cells stained with biotinylated antibodies, add 12.5 μl/ sample streptavidin-PE (1:100 dilution)
- Incubate .5 hours on ice
- Wash cells with 1 ml DPBS (plain) two times, spin, dump, rack
- To fix, add 0.25 ml 2 % paraformaldehyde